REMARKS

Rejections for double-patenting

Claims 1-5, 8, 10-12, 14-16 and 20-23 have been rejected under the judicially created doctrine of obviousness-type double patenting as being obvious over claims 1, 2, 4-7, 12-13, and 15-19 of U.S. Pat. No. 5,952,176 (hereinafter referred to as "U.S. '176") in view of Chirikjian et al., U.S. Pat. No. 5,656,430 (hereinafter referred to as U.S. '430). Applicants traverse this rejection and withdrawal thereof is respectfully requested.

The Examiner asserts that the indicated claims of U.S. '176 encompass a method of rapidly detecting the presence or absence of a particular nucleic acid at a candidate locus. U.S. '176 is asserted to differ from the present invention only by failing to form an upstream DNA fragment by cleaving at an abasic site and extending the fragment. U.S. '430 is asserted to teach a method of detecting a point mutation by cleaving a 5′ probe and binding it to a template for DNA polymerase with dNTP. Applicants traverse this rejection and withdrawal thereof is respectfully requested.

The Examiner asserts that U.S. '430 teaches a method of detecting a point mutation by cleaving a 5′ probe and binding it to a template for DNA polymerase with dNTP. However, this is a mischaracterization of the reference. U.S. '430 may be summarized as teaching the following.

1) U.S. '430 teaches the use of a template and polymerase extension, i.e. that DNA containing a 3' OH terminus can be extended with polymerase and/or ligase enzymes using a template to direct the extension. This feature was well known in the art.

2) U.S. '430 further teaches that a probe (specifically a synthetic probe) is annealed to target DNA to form a mismatch. Cleavage of the probe only occurs if a mismatch is generated. As a result, a set of at least two probes would have to be used each time to study the site of the polymorphism. With the present invention, on the other hand, no probe is added to generate the upstream fragments. In addition, all upstream fragments (for example, two upstream fragments that are indicative of a bi-allelic polymorphism) can be generated from the same reaction.

3) Finally, U.S. '430 teaches that the probe binds to the target nucleic acid, is cleaved and subsequently extended. Thus, U.S. '430 differs from the present invention wherein upstream fragments are generated by first amplifying the target nucleic acid and then cleaving it to form the extendible upstream fragments. The upstream fragments are extended using a separate template, which is distinct from the original target nucleic acid. This feature results in one of the advantages associated with the present invention. With the present invention one of the upstream fragments can be selected out of mixture of upstream fragments. As

a result, one can differentially and specifically extend each upstream fragment in the mixture, allowing for the simultaneous detection of all alleles of a particular polymorphism of interest and/or any number of polymorphisms of interest.

The Examiner interprets U.S. '430 as teaching the formation of a DNA fragment by the upstream cleavage of the DNA at an abasic site followed by extension. However, the interpretation of U.S. '430 by the Examiner, mischaracterizes the reference. U.S. '430 teaches that their probe is cleaved at a site of a mismatch by a DNA repair enzyme and then extended on that same template strand or denatured and extended by DNA terminal transferase, which is template independent.

The method of the present invention, on the other hand, comprises the steps of: i) introducing a modified base which is a substrate for a DNA glycosylase into a DNA molecule; ii) excising the modified base with DNA glycosylase to generate an abasic site; iii) cleaving the DNA at the abasic site to generate and release an extendible upstream DNA fragment having a 3' hydroxyl terminus; and iv) incubating the released extendible upstream DNA fragment in the presence of an enzyme for the extension thereof and an additional template nucleic acid and analysing resultant fragment(s).

The extension reaction of the present invention differentiates between various upstream fragments that are produced, whereas the extension reaction in U.S. '430 is only used as a means to label the fragments generated in the mismatch reaction.

Claim 1 has been amended to more clearly recite the differences between U.S. '430 and the present invention. Specifically, claim 1 has been amended to explicitly recite that an "additional" template nucleic acid is used. Support for this amendment is found in the specification on page 21, line 1-14 and the Examples. As discussed above, with U.S. '430 the probe is cleaved at a site of a mismatch by a DNA repair enzyme and then extended on that same template strand. Claim 1 has been further amended to more clearly recite that cleavage of the DNA at the abasic site generates and releases the extendible upstream DNA fragment having a 3' hydroxyl terminus. With U.S. '430, the 5' end of the cleaved product "remains bound" to the target. Thus, with the present invention, unlike U.S. '430, the "template" in step iv) is different from the template/target from which it is generated. These amendments to claim 1 do not narrow these features of the invention because these features were already inherently present in original claim 1. The amendments explicitly recite features already present.

Thus, U.S. '430 fails to teach that an upstream fragment is formed by cleaving the DNA at an abasic site and extending it, as required in the present invention. For example, the reaction of U.S. '430 fails to use an additional template or to release the cleaved product. As a result if U.S. '430 is combined with U.S. '176 the present invention is not achieved. The rejection for obviousness-type double-patenting is therefore respectfully requested.

Objections to the specification

The specification has been objected to for failing to contain an Abstract of the Disclosure. The specification has been amended as indicated above, to include an Abstract. No new matter has been added with the Abstract. Withdrawal of the objection is respectfully requested.

Objections to the claims

Claim 22 has been objected to as being improperly dependent. Claim 22 has been cancelled, thus obviating this rejection.

Rejections under 35 U.S.C. §112, second paragraph

Claims 1-23 have been rejected under 35 U.S.C.§112, second paragraph as being indefinite. More specifically, claim 1 has been

rejected for recitation of "a DNA molecule." The Examiner indicates that it is not clear whether the DNA molecule is single or double stranded. Applicants traverse this rejection and withdrawal thereof is respectfully requested. As discussed on page 6, line 22 through page 7, line 28 and page 11, lines 6-20 the DNA molecule used in the present method may be either single or double stranded. As such, the metes and bounds of the claim are clearly defined by recitation of "a DNA molecule" and withdrawal of the rejection is respectfully requested.

The Examiner additionally indicates that recitation of "the extendible upstream fragment" and "the resultant fragment" lack antecedent basis. Claim 1 has been amended to provide proper antecedent basis for all elements. Withdrawal of the rejections is therefore respectfully requested.

Claim 13 has been rejected as being vague in the recitation of "wherein one or more of the nucleotide(s) of step iv) is a dideoxy nucleotide" because of a lack of antecedent basis. Claim 14 has been rejected as being vague in the recitation of "wherein one or more of the nucleotide(s) of step iv) is labeled" because of a lack of antecedent basis. Claim 12 has been amended to provide antecedent basis for the recitation of step iv) in claims

13 and 14. Withdrawal of the rejections is therefore respectfully requested.

Claim 19 has been rejected as being unclear in the meaning of "the reporter oligonucleotide is partially degenerate." Applicants traverse this rejection and withdrawal thereof is respectfully requested. The terms "degenerate" and "partially degenerate" are common terms of art in the field of the present invention. "Degeneracy" means that any one or more of the nucleic acid bases in the oligonucleotide can be replaced with one or more of the other three bases. For example, if an oligonucleotide has a four-fold degeneracy at position "X," then 25% of a solution containing the oligonucleotide will have "G" at position "X," 25% will have "C," 25% will have "A," and 25% will have "T." An oligonucleotide may have degeneracy at all or only a portion (i.e. partial) of the bases and at each of the degenerate positions, the degeneracy may be 2-, 3-, or 4-fold. A 2- or 3-fold degeneracy at a particular position is also referred to as "partial" degeneracy.

The terms "reporter molecule" and "partially degenerate" are also discussed in the specification at page 18, lines 14-17 and at page 22, line 31 through page 23, line 6. Examples of reporter oligonucleotides are presented in Example 4 and illustrated in Figure 7. As such, the meaning of the phrase "the reporter

oligonucleotide is partially degenerate" is clear and withdrawal of the rejection is respectfully requested.

Rejections under 35 U.S.C.§103

Claims 1-2 and 8-23 have been rejected under 35 U.S.C. §103 as being obvious over McCarthy et al., WO 97/03210 (hereinafter referred to as "WO '210") in view of U.S. '430. WO '210 is asserted to differ from the present invention only in failing to disclose extending an upstream fragment with a template and incubating the extendible upstream fragment with ligase in the presence of a reporter oligonucleotide. U.S. '430 is asserted to teach a method of detecting a point mutation by cleaving a 5' probe and binding it to a template for DNA polymerase with dNTP.

The Examiner asserts that one skilled in the art would have been motivated to combine WO '210 with U.S. '430 because the method of WO '210 is used to rapidly and easily detect multiple known mutations in DNA and the method of U.S. '430 is efficient and sensitive through the use of a probe with labeled nucleotides as a signal.

WO '210 is the International Application corresponding to U.S. '176 discussed above. As such, the arguments presented above under the "Rejections for double-patenting" are equally applicable to the present rejection. As discussed above, U.S. '430 fails to teach that an upstream fragment is formed by cleaving the DNA at an

abasic site and extending it, as required in the present invention. For example, the reaction of U.S. '430 fails to use an additional template or to release the cleave product. As a result if U.S. '430 is combined with WO '210 the present invention is not achieved. Withdrawal of the rejection is therefore respectfully requested.

Claims 3-7 have been rejected as being obvious over WO '210 combined with U.S. '430 and Dianov et al. Further to the asserted teachings of WO '210 and U.S. '430 discussed above, Dianov is asserted to teach the use of 5' AP endonuclease and a 5' deoxyribophosphodiesterase to treat apurinic and apyridimic sites. Further to the arguments above regarding WO '210 and U.S. '430, Dainov et al. further fails to teach that an upstream fragment is formed by cleaving the DNA at an abasic site and extending it, as required in the present invention. As such, Dianov et al. fails to teach the deficiencies of WO '210 and U.S. '430 and the present invention is not achieved by combining the references. Withdrawal of the rejection is therefore respectfully requested.

Applicants note that the subject matter of all of the claims of the present application were commonly owned at the time of invention.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact MaryAnne Armstrong, PhD (Reg. No. 40,069) at the telephone number of the listed below.

A marked-up version of the amended claim showing all changes is attached hereto.

Pursuant to 37 C.F.R. §§ 1.17 and 1.136(a), Applicant(s) respectfully petition(s) for a two (2) month extension of time for filing a reply in connection with the present application, and the required fee of $200.00 is attached hereto.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

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Attachments

VERSION WITH MARKINGS TO SHOW CHANGES MADE

1. (Amended) A method for characterising nucleic acid molecules, which comprises the steps of:

i) introducing a modified base which is a substrate for a DNA glycosylase into a DNA molecule;

ii) excising the modified base by means of said DNA glycosylase so as to generate an abasic site;

iii) cleaving the DNA at the abasic site so as to generate **and release** an **extendible** upstream DNA fragment **having a 3' hydroxyl terminus** ~~that can be extended~~; and

iv) incubating the **released** extendible upstream **DNA** fragment in the presence of an enzyme allowing for extension thereof and ~~a~~ **an additional** template nucleic acid and analysing ~~the~~ resultant fragment(s).

12. (Amended) A method according to Claim 11, wherein the extendible upstream fragment is incubated **in step iv)** with the polymerase in the presence of one or more nucleotide(s).